Application No. 09/580,476

Within the amended claims, claims 5, 21-23, 33-34, and 54-65 have been amended in consideration of the indication of their allowable nature. Although not initially included within the list of allowable claims, the remaining amended claims have been amended in order to provide them in a form dependent on various allowable claims. Hence these claims should raise no new issues, having been previously pending and considered, and are patentable for the reasons that underlay the patentability of the claims from which they depend, and for others as well. Upon entry of this amendment, claims 2-5, 7-9, 15-25, 33-42 and 49-65 will be pending and in condition for allowance.

Entry of this Amendment and early allowance of the pending claims is respectfully requested.

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## VERSION WITH MARKINGS TO SHOW CHANGES MADE

Amendments to the claims (where insertions are underlined and deletions placed in brackets):

- 2. (Once Amended) A gene expression and delivery system according to claim [1] 21 wherein the replicon of [flavivirus] Kunjin virus origin includes the nucleotide sequence for a [flavivirus] Kunjin virus 5' untranslated region (UTR), at least a portion of the 5' coding region for [flavivirus] Kunjin virus core protein, the nucleotide sequence coding for the [flavivirus] Kunjin virus nonstructural proteins, and part or all of the 3'-terminal sequence of a [flavivirus] Kunjin virus 3'UTR, required for self-replication of [flavivirus] Kunjin virus genomic material, which vector is adapted to receive at least a nucleotide sequence without disrupting its replication capabilities.
- 3. (Once Amended) A gene expression and delivery system according to claim [1] 21 wherein the replicon contains a sufficient amount of [flavivirus] Kunjin virus 5' UTR and a sufficient amount of 5' [flavivirus] Kunjin virus coding region for core protein required for RNA replication.
- 4. (Once Amended) A gene expression and delivery system according to claim [1] 21 wherein the replicon contains a [flavivirus] <u>Kunjin virus</u> 5' UTR and at least about between 60 and 80 nucleotides from the 5' coding region for [flavivirus] <u>Kunjin virus</u> core protein.
- 5. (Once Amended) A gene expression and delivery system according to claim [1] 21 wherein the replicon [is derived from Kunjin virus and] contains the Kunjin virus 5' UTR and at least 60 nucleotides of the Kunjin virus 5' core protein coding region.
- 7. (Once Amended) A gene expression and delivery system according to [1] <u>21</u> wherein the replicon is adapted to receive at least a nucleotide sequence at any point in the replicon that does not effect processing of [flavivirus] <u>Kunjin virus</u> proteins and RNA replication.

- 15. (Once Amended) A gene expression and delivery system according to [1] <u>21</u> wherein the replicon includes after the 3' terminal sequence of a [flavivirus] <u>Kunjin virus</u> 3'UTR a sequence cassette containing antigenomic ribozyme of the hepatitis delta virus and SV 40 polyadenylation signal, required for production of vector RNA with precise 3'terminus possessing high replication efficiency.
- 16. (Once Amended) A gene expression and delivery system according to claim [1] 21 wherein the replicon is an RNA based vector.
- 18. (Once Amended) A gene expression and delivery system according to claim [1] 21 wherein the replicon is a DNA based vector.
- 19. (Once Amended) A gene expression and delivery system according to claim [1] 21 wherein the replicon is a DNA based vector, which is capable of producing replicon RNA in cells by cellular DNA-dependent RNA polymerase from plasmid DNA incorporating mammalian expression promoters preceding the replicon sequence.
- 20. (Once Amended) A gene expression and delivery system according to claim [1] 21 wherein the replicon is derived from a single [flavivirus] <u>Kunjin virus</u> species.
- 21. (Once Amended) A gene expression and delivery system comprising:
  - a replicon of flavivirus origin as a first vector, which is adapted to receive at least a nucleotide sequence without disrupting its replication capabilities and which is unable to express at least part or all of a structural protein(s) region and or a protein(s) or part thereof required for packaging of a flavivirus genome into a virus-like particle; and
  - (b) at least a second vector that is capable of expressing flavivirus structural protein(s) and/or any other proteins required for packaging of the replicon into infectious flavivirus-like particles, [A gene expression and delivery system according to claim 1] wherein the replicon is derived from Kunjin virus.

- 24. (Once Amended) A gene expression and delivery system according to claim [1] <u>21</u> wherein the second vector is heterologous in origin to the origin of the replicon.
- 25. (Once Amended) A gene expression system according to claim [1] <u>21</u> wherein the second vector is derived from an alphavirus.
- 33. (Once Amended) A gene expression system according to claim [1] <u>21</u> wherein [the replicon is derived from Kunjin virus and] the second vector is derived from Semliki Forest Virus.
- 34. (Once Amended) A gene expression system according to claim [1] <u>21</u> wherein [the replicon is derived from Kunjin virus and] the second vector is derived from Sindbis virus.
- 35. (Once Amended) A gene expression system according to claim [1] <u>21</u> wherein the replicon is adapted to include part or all of the following: at least, about the first 150 nucleotides of a [flavivirus] <u>Kunjin virus</u> genome; at least about the last 60 nucleotides of E protein; substantially all of the nonstructural region; and part or all of the 3'UTR.
- 36. (Once Amended) A gene expression system according to claim [1] <u>21</u> wherein the replicon is adapted to include part or all of the following: the first 157 nucleotides of the [KUN] <u>Kunjin virus</u> genome, the last 66 nucleotides of E protein, the entire nonstructural region, and all of the 3'UTR.
- 37. (Once Amended) A gene expression system according to claim [1] <u>21</u> wherein the replicon encodes all [flavivirus] <u>Kunjin virus</u> structural proteins except for [flavivirus] core protein and the second vector is SFV-C.

- 38. (Once Amended) A gene expression system according to claim [1] <u>21</u> wherein the replicon encodes [flavivirus] <u>Kunjin virus</u> core protein and the second vector is SFV-prME as herein described.
- 39. (Once Amended) A gene expression system according to claim [1] <u>21</u> wherein the replicon does not encode any [flavivirus] <u>Kunjin virus</u> structural proteins and the second vector is SFV-prME-C and SFV-prME-C105 [as herein described].
- 49. (Once Amended) A DNA based replicon vector according to claim [48] <u>65</u> wherein the complementary DNA sequence of the nucleotide sequence includes a [flavivirus] <u>Kunjin virus</u> 5' untranslated region (UTR), at least a portion of the 5' coding region for [flavivirus] <u>Kunjin virus</u> core protein, the nucleotide sequence coding for the [flavivirus] <u>Kunjin virus</u> non-structural proteins, and part or all of the 3'-terminal sequence of a [flavivirus] <u>Kunjin virus</u> 3'UTR, required for self-replication of [flavivirus] <u>Kunjin virus</u> genomic material, which vector is adapted to receive at least a nucleotide sequence without disrupting its replication capabilities.
- 50. (Once Amended) A DNA based replicon vector according to claim [48] <u>65</u> wherein the eucaryotic expression promoter is a cytomegalovirus early enhancer promoter region.
- 51. (Once Amended) A DNA based replicon vector according to claim [48] <u>65</u> wherein the replicon includes at its 3'end an antigenomic ribozyme of hepatitis delta virus and simian virus 40 polyadenylation signal cassette, required for production of efficiently replicating replicon RNA with the precise 3'terminus.
- 52. (Once Amended) A DNA based replicon vector according to claim [48] <u>65</u> wherein the replicon contains a sufficient amount of the complementary DNA sequence of the [flavivirus] <u>Kunjin virus</u> 5' UTR and the 5' [flavivirus] <u>Kunjin virus</u> coding region for core protein required for RNA replication.

- 53. (Once Amended) A DNA based replicon vector according to claim [48] <u>65</u> wherein the replicon contains the complementary DNA sequence of a [flavivirus] <u>Kunjin virus</u> 5' UTR and at least about between 60 and 80 nucleotides from the 5' coding region for [flavivirus] <u>Kunjin virus</u> core protein.
- 54. (Once Amended) A DNA based replicon vector according to claim [48] <u>65</u> wherein the replicon [is derived from Kunjin virus and] contains the complementary DNA sequence of the Kunjin virus 6' UTR and at least 60 nucleotides of the Kunjin virus 5' core protein coding region.
- (Once Amended) A DNA based replicon vector according to claim [48] 65 wherein the replicon includes the complementary DNA sequence of the nucleotide sequence for the [flavivirus] Kunjin virus 5'UTR, at least a portion of the 5' nucleotide coding region for [flavivirus] Kunjin virus core protein, the nucleotide coding region for [flavivirus] Kunjin virus nonstructural proteins, a sufficient amount of the 3'-terminal region of the [flavivirus] Kunjin virus 3'UTR required for self-replication of [flavivirus] Kunjin virus genomic material wherein (i) the vector is adapted to receive at least a nucleotide sequence without disrupting the replication capabilities of the vector, (ii) the nucleotide sequence is inserted into the vector in a manner which deactivates expression of at least a gene that would otherwise code for a [flavivirus] Kunjin virus structural protein and (iii) the inserted nucleotide sequence does not encode for the structural protein sequence that it deactivates.
- 56. (Once Amended) A DNA based replicon vector according to claim [48] <u>65</u> wherein the replicon is adapted to receive at least a nucleotide sequence at any point in the replicon that does not effect processing of [flavivirus] <u>Kunjin virus</u> proteins and RNA replication.
- 57. (Once Amended) A DNA based replicon vector according to claim [48] <u>65</u> wherein the nucleotide sequence is inserted into the 3 UTR of the replicon.

- 58. (Once Amended) A DNA based replicon vector according to claim [48] <u>65</u> wherein the nucleotide sequence that is inserted into the 3 UTR of the replicon is preceded by an IRES sequence.
- 59. (Once Amended) A DNA based replicon vector according to claim [48] <u>65</u> wherein the nucleotide sequence is inserted within a structural gene.
- 60. (Once Amended) A DNA based replicon vector according to claim [48] <u>65</u> wherein the nucleotide sequence is inserted in place of at least a deleted structural gene.
- 61. (Once Amended) A DNA based replicon vector according to claim [48] <u>65</u> wherein the nucleotide sequence that is inserted in place of deleted structural proteins of the replicon is followed by a termination codon and a IRES sequence.
- 62. (Once Amended) A DNA based replicon vector according to claim [48] <u>65</u> wherein the inserted nucleotide sequence possesses at its 3' end a 2A autoprotease sequence of foot and mouth disease virus.
- 63. (Once Amended) A DNA based replicon vector according to claim [48] <u>65</u> wherein the inserted nucleotide sequence possesses at its 5'end a mouse ubiquitin sequence.
- 64. (Once Amended) A DNA based replicon vector according to claim [48] <u>65</u> wherein the replicon is derived from a single [flavivirus] <u>Kunjin virus</u> species.
- 65. (Once Amended) A DNA based replicon vector of Kunjin virus origin, wherein the vector comprises:
  - (a) a complementary DNA sequence that is adapted to receive at least a nucleotide sequence without disrupting its replication capabilities and which is unable to express at least part or all of a structural protein(s) region and or a protein(s) or

- part thereof required for packaging of a Kunin virus genome into a virus-like particle;
- (b) a mammalian expression promoter 5' to the complementary DNA sequence of Kunjin virus origin; and
- (c) at least a second nucleotide sequence capable of terminating transcription of replicon RNA with a precise 3' terminus; and wherein the promoter and the second nucleotide sequence are capable of promoting transcription and terminating same, of Kunjin virus RNA within the nucleus of a transfected cell [according to claim 48 wherein the replicon is derived from Kunjin virus].

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